METABOLISM OF NUCLEIC ACID COMPONENTS IN BACTERIA[1]

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INTRODUCTION

I should like to express my appreciation for the privilege of addressing the Society of American Bacteriologists and of discussing some of the problems that we have considered and the conclusions reached during the last few years in our study of the manner in which certain of the component parts of the nucleic acid molecule are utilized by bacteria. It would be only proper for me to express first my appreciation to the many colleagues who have cooperated with me in these ventures. I feel that I owe a double debt to them: First, it has been my impression that ideas are conceived and clarified best during discussion, when one is forced to explain and defend concepts and interpretations and particularly to support his suggestions for the next experiment. The integration of ideas certainly appears to be catalyzed by this interplay of opinions. Secondly, research has been a pleasurable vocation and as such has inspired an urge to tell others about it. I can only thank my colleagues for their forbearance in listening to what must have been over-enthusiastic and repetitive resumés of experimental results.

At a time such as this, one almost involuntarily is lead to a consideration of the basic importance of his field of research. The reasons why biologists are interested in the metabolism of nucleic acids are obvious and have been discussed so frequently as to warrant little amplification here. The importance of desoxyribonucleoproteins as hereditary determinants is apparent to all, although the exact extent and nature of their role remain a subject for debate. Similarly many types of evidence have indicated an interrelation between ribonucleic acids and protein synthesis, especially under conditions of adaptive enzyme formation. The possibility that a nucleic acid derangement produces in some manner the uncontrolled growth characteristic of malignant cells has also attracted many. For the bacteriologist the discovery that the smallest infectious units, the viruses, consist in major part of nucleic acids has led to a heightened realization of the necessity of a more complete understanding of the metabolism of these substances as a guide to rational therapy.

In addressing this audience it is not necessary to explain why one chooses microorganisms for a study of the nucleic acids. There are, however, several aspects of this question that I should like to consider for a moment. In the first place, our knowledge of the place of bacteria in the nitrogen cycle as it concerns the nitrogenous bases of the nucleic acids is scant. We have some idea of the manner in which purines can be decomposed, either oxidatively or fermentatively,

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although for each of these processes our concepts are extremely fragmentary. It is only within the last few years that the action of bacteria in degradation of pyrimidines has been investigated. I shall discuss these results briefly in a later portion of this address. The general advantages of microorganisms for metabolic studies apply to work with the nucleic acids as well: *i.e.*, the unusual adaptability of the experimental material, the opportunity of defining growth conditions in ways not possible with a homeostatic animal, and the simplification of the intercellular interactions. In addition, most workers have observed that the rate of nucleic acid synthesis in a bacterial cell, which can readily be obtained in a stage of rapid cell division, is considerably greater than in the relatively more dormant animal cell. Thus, one frequently obtains a considerable enrichment of the desired activity by the use of the bacterial system.

DESIGN OF CHEMOTHERAPEUTIC AGENTS

A primary practical goal for an investigator of the interaction of nucleic acid components is the eventual therapeutic application of his observations, that is, in general, the design of successful chemotherapeutic agents. It is obvious that an essential characteristic of any such agent is selectivity. It must *aid* the host in its battle with the microorganism, by killing the invader, retarding its growth, or preventing the deleterious effects of its products. *A priori*, one would consider a substance which prevented nucleic acid synthesis an excellent possibility as such a selective agent. The consequences of a short period of inability to form nucleic acids should certainly be much less serious for the nondividing animal cell than for the invader which must multiply rapidly, else it be eliminated by the defenses of the host.

Our present knowledge of the manner in which sulfonamides affect the metabolism of the cell will illustrate this selectivity. The discovery by Woods (31) that *p*-aminobenzoic acid (PAB) competitively antagonized the inhibitory action of the sulfonamides, and the subsequent demonstration that a variety of bacteria require PAB for growth, suggested that this substance plays an important role in cellular metabolism, this role being impaired in the presence of sulfonamides.

When structural studies on folic acid indicated it to be a derivative of PAB, an investigation of the sulfonamide-folic acid interrelations became inviting. We found (15, 16) that bacteria can be divided into three groups on the basis of their response to folic acid.

(I) Certain lactobacilli and streptococci require preformed pteroylglutamic acid for growth. These organisms, growing in the presence of small amounts of pteroylglutamic acid, are virtually insensitive to the sulfonamides.

(II) Certain closely related organisms can synthesize their own pteroyl compounds, and under these conditions they are extremely sensitive to sulfonamides. When quantities of pteroylglutamic acid sufficient for growth of the pteroyl-dependent group (Type I) are present, the organisms are unaffected by sulfonamides. This action of pteroyl compounds suggests a metabolic basis for the known clinical resistance of infections with the group D streptococci to sulfonamide therapy.

(III) With a variety of other organisms (including certain pneumococci, staphylococci, and all gram-negative organisms tested) the addition of pteroylglutamic acid does not counteract sulfonamide inhibition of growth, although with certain of these organisms it has been possible to demonstrate that the addition of sulfonamides prevents the formation of a substance with the biological activity of folic acid. One may suggest, therefore, that in all organisms the sulfonamides block the conversion of PAB to pteroyl compounds but that the product of this synthesis is not pteroylglutamic acid itself. Only a relatively few organisms (including man) are able to convert pteroylglutamic acid to this functional form.

Purines, thymine, methionine and certain other amino acids also antagonize sulfonamides in a manner which indicates them to be products of the catalytic action of this functional form of pteroylglutamic acid. Biochemical studies of the past ten years have provided ample corroborative evidence for this concept. Additional information was obtained with a PAB-requiring mutant of *Escherichia coli* (17). This organism grew either in the presence of PAB alone or of a combination of a purine, thymine, and a mixture of amino acids. The mutant (and the parent strain of *E. coli*) was insensitive to sulfonamides when growing in the presence of the purine-thymine-amino acid mixture, whereas it was highly sensitive when growing on PAB and hence required to synthesize its own supplies of these substances. Thus the mutant resembles a sulfonamide-inhibited organism, with formation of the functional pteroyl compound prevented *in the mutant* by its inability to *synthesize* PAB and in the sulfonamide-inhibited organism by its inability to convert PAB to the active coenzyme.

One cannot be certain that the therapeutic action of a sulfonamide in any given instance is the result of the interactions just described. Such proof may well necessitate additional detailed information on the manner and reactions in which this functional form (or forms) acts. The point I should like to emphasize is that in the sulfonamides we possess compounds with which we can not only prevent the synthesis of nucleic acids by the bacterial cell, but can accomplish this by blocking formation of a coenzyme essential for this purpose. The microorganism should show a maximal sensitivity to the inhibitor under these conditions, particularly when, as with the pteroyl compounds, many cells do not appear to utilize external sources of the coenzyme.

How then is one to develop other compounds with potential therapeutic value? Many avenues of approach exist, but an attempt to interfere with the process of nucleic acid synthesis remains one of the most promising. Here certainly there is a possibility of achieving an inhibitor with the desired combination of selectivity and high activity. If this approach is to be a logical one it must, however, be based on a better understanding than we now possess of the manner in which nucleic acids are synthesized and degraded. On the basis of this belief, I have turned during the past five years to a study of these processes. While much may be accomplished by experimentation at the level of complexity of the nucleic acid polymer, it appeared more profitable and experimentally simpler to define the pathways by which some of the components of the eventual polymer are metabolized. I am convinced that such experimentation will provide the

techniques and concepts essential for an approach to the synthesis of the nucleic acid itself.

CLEAVAGE AND INTERCONVERSION OF NUCLEOSIDES

We have considered first the action of bacterial cells on the pentose and desoxypentose nucleosides. Figure 1 illustrates some of the reactions which have been demonstrated with the ribose nucleosides (inosine is used as an example). The nucleoside can undergo phosphorolytic cleavage to yield the free base (hypoxanthine in this instance) and ribose-1-phosphate which is converted to ribose-5-phosphate. Degradation of the ribose-5-phosphate then occurs, probably *via* ribulose phosphate. In the presence of arsenate, cleavage of the nucleoside presumably yields the 1-arsenate ester which decomposes spontaneously, giving rise to free ribose. The nucleosidase reaction can often be conveniently followed by the use of this system.

With the desoxyribose nucleosides we demonstrated the formation of desoxyribose-5-phosphate during cleavage of hypoxanthine desoxyriboside (22), and

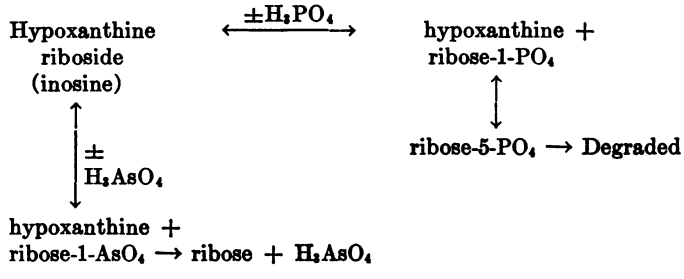

FIG. 1. Degradation of ribose nucleosides

Friedkin and Kalckar (5) have isolated desoxyribose-1-phosphate. Here, as with the pentoses, degradation of the carbon chain appears to take place by cleavage of the 5-phosphate ester. Most of these reactions were first observed in animal tissue but have, in general, been readily demonstrated in bacterial cells as well.

We have studied these conversions mainly in *Escherichia coli* which is a rich source of the enzymes concerned. Cleavage of both purine and pyrimidine desoxyribose nucleosides has been obtained, and in each instance the formation of the desoxypentose phosphate could be demonstrated (23). When thymine and desoxyribose-1-phosphate were incubated with an extract of *E. coli*, thymidine is formed in the expected amounts, thus the reaction is clearly reversible. This reversibility is also evident from the experiments presented in figure 2. This represents chromatograms of aliquots of the incubation mixtures after varying intervals of time. In Experiment A, hypoxanthine desoxyriboside (HXDR) was incubated with thymine in the presence of the enzyme. Within one and a half hours hypoxanthine and thymine desoxyriboside had formed, presumably by means of an initial phosphorolysis of the hypoxanthine nucleoside and combination of the resulting desoxyribose-1-phosphate with thymine forming the thymine nucleo-

side. The reverse reaction is shown in Experiment B, where hypoxanthine and the thymine nucleoside yield free thymine and the hypoxanthine nucleoside. A similar exchange between hypoxanthine desoxyriboside and uracil is shown in Experiment C. Thus the organism possesses a means by which interconversion of nucleosides can occur in the presence of inorganic phosphate.

After the initial demonstration that the purine nucleosidase of liver, thought to be a hydrolytic enzyme, was in fact a nucleoside phosphorylase (14), it seemed probable that most nucleosidases acted in this manner. Recently, however, several investigators have reported that direct hydrolytic cleavage of the nucleosides can occur, particularly with preparations from microorganisms. Carter (1), for instance, found a hydrolytic uridine nucleosidase in yeast. We reported that *Lactobacillus pentosus* contains a nucleosidase catalyzing hydrolysis of ribosides

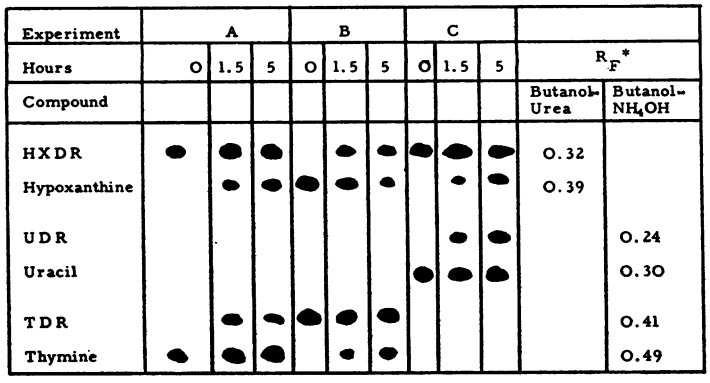

FIG. 2. Desoxyribose nucleoside interconversion

The substrates were incubated with enzyme preparations obtained by grinding cells of *Escherichia coli* with alumina and extracting the paste with phosphate buffer. At the indicated times aliquot samples were heated and chromatographed. The figure is a composite diagram of the results obtained with the two solvent systems.

(18), and Heppel and Hilmoe (9) have demonstrated in bakers' yeast a hydrolytic enzyme which cleaved a large number of purine nucleosides.

A third type of nucleoside interconversion was discovered by MacNutt (21). He observed the transfer of desoxyribosyl (DR-) units from one nitrogeneous base to another in extracts of *Lactobacillus helveticus*. Phosphate was not required nor did desoxyribose or desoxyribose-1-phosphate participate in the reaction. *E. coli* contains similar DR-transferring enzymes (10); they appear to be, however, more specific than those of *L. helveticus*. In a partially purified extract, free of significant quantities of phosphate, transfer of DR- units occurs from one pyrimidine to another or from one purine to another, but does not occur between purines and pyrimidines unless inorganic phosphate is added. By fractionation of these extracts it has been possible to separate the DR-transferring enzymes from the nucleosidases and the purine nucleosidase from the pyrimidine enzyme (11).

We are inclined, therefore, to represent the reactions in *E. coli* as shown in figure 3. I have indicated two DR-transferring enzymes, one transferring DR- from thymine to uracil (and the reverse) by means of a DR-enzyme complex. Whether or not other pyrimidines can react here is not known. The other enzyme reacts with hypoxanthine or with guanine. Again, reaction with other purines has not been studied. In the absence of inorganic phosphate or arsenate, these are the only reactions that occur. If phosphate is added, phosphorolysis of either type of nucleoside can occur, and the desoxyribose-1-phosphate reacts with the other nucleosidase and the opposite type of base to yield a new nucleoside as in the chromatographic experiments presented previously. When the nucleosides are incubated with these extracts, acid-soluble desoxyribose does not disappear unless inorganic phosphate is added (11); hence formation of the phosphate esters is apparently necessary before degradation of the carbon chain can occur. Free desoxyribose is not degraded either by the extracts or by intact cells, and the

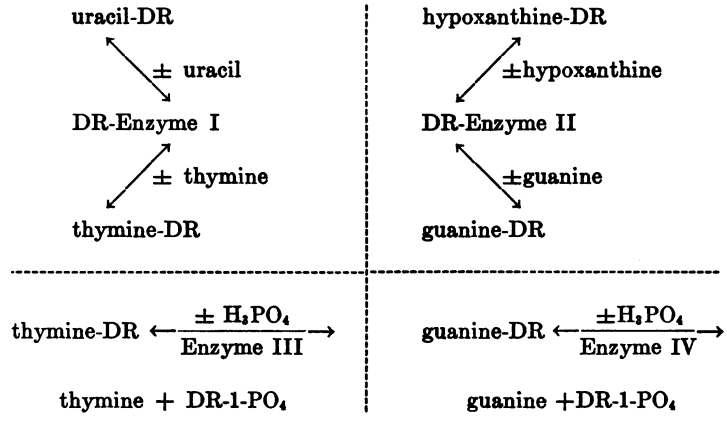

FIG. 3. Mechanism of nucleoside interconversion in *Escherichia coli*

addition of arsenate during degradation of desoxyribose nucleosides by the intact cell leads to the formation of free desoxyribose. This would be expected from arsenolysis of the nucleosides.

The products of desoxyribose nucleoside fermentation in *E. coli* have been determined in our laboratory by Hoffmann and Manson (12). Their results may be summarized by the equation:

$$\text{Thymidine} \rightarrow \text{Thymine} + CH_3COOH + CH_3CH_2OH + HCOOH \text{ (or } H_2 + CO_2).$$

Thus two C_2 units and a C_1 unit have been formed from the C_5 chain. Since Racker (25) has recently demonstrated with extracts of *E. coli* the condensation of triose phosphate and acetaldehyde to yield desoxyribose-5-phosphate, we can present with reasonable confidence the scheme for thymidine fermentation shown in figure 4. Thymidine yields first thymine and desoxyribose-1-phosphate, which is converted to the 5-phosphate ester. This ester is cleaved to triose phosphate and acetaldehyde. The acetaldehyde is reduced to ethanol; the triose phosphate

is converted to pyruvic acid by the usual glycolytic pathway, and pyruvate then is cleaved to acetate and formate. As these cells contain an active formic hydrogenlyase, it would be expected that a part of the formate would accumulate as H_2 and CO_2. This formulation implies an initial cleavage of the C_6 chain into C_2 and C_3 units with cleavage occurring between carbons–2 and –3 of the chain.

DECOMPOSITION OF PENTOSE DERIVATIVES

Let us turn now to the fate of the pentose derivatives formed during the nucleoside cleavage. In searching for a system in which to study the degradation of pentose compounds, we selected the fermentation of pentoses by homofermentative lactic acid bacteria as being probably the simplest. About 30 years ago, Fred, Peterson and collaborators (3, 4) reported that these organisms degraded pentoses with the formation of equimolar quantities of acetic and lactic acids. They suggested a direct cleavage of the C_6 chain of the pentose into C_2 and C_3 units. As a further test of this hypothesis we prepared xylose labelled with C^{14}

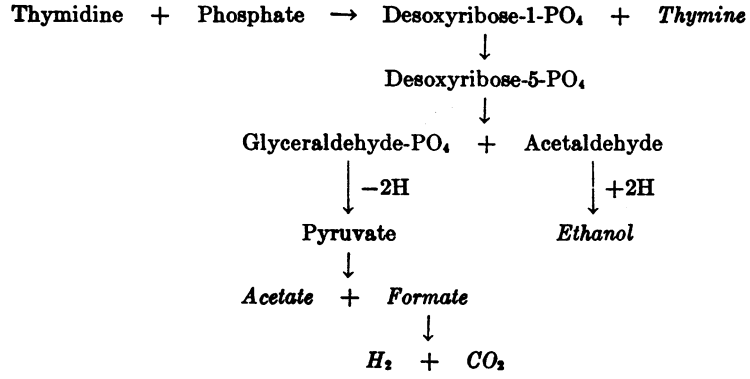

FIG. 4. Suggested pathway of thymidine degradation in *Escherichia coli*

in carbon–1, the aldehyde carbon, and incubated this material with washed resting cells of *L. pentosus* grown on xylose (6). Of the products, only the acetic acid contained significant amounts of C^{14}, and all of this C^{14} was present in the methyl group. Thus the methyl group of the acetic acid was derived from the aldehyde carbon of xylose, and we presume that carbon–2 of xylose is the precursor of the acetic acid carboxyl and that carbons –3, –4 and –5 are converted to lactic acid (figure 5).[2] These results offer strong support for the hypothesis of cleavage into C_2 and C_3 fragments and indicate that cleavage occurred between carbons –2 and –3 as with the desoxypentose chain. To explain the formation of acetic acid labelled only in the methyl group, it appears most logical to suggest that a 2-ketopentose is formed as an intermediate. Cleavage of this

[2] Dr. I. A. Bernstein recently obtained biologically-labelled samples of ribose and has degraded them by fermentation with *L. pentosus* and by chemical procedures. His results confirm our suggestion as to the fate of the other carbons of the pentose chain. (Personal communication.)

molecule would yield a C_2 compound with the C^{14} in the more reduced group, and this group might then be converted to the methyl group of the acetic acid. Glycolaldehyde is indicated here as the C_2 product merely in an illustrative manner. This substance *per se* does not appear to be an intermediate in this reaction. It is not converted to acetic acid by cells which can readily form acetic acid from xylose. However, the actual substance involved must be closely related to glycolaldehyde, perhaps a bound form.

Evidence for the occurrence of a similar mechanism in a heterofermentative lactic acid organism was obtained by Rappoport *et al.* (26). They studied the fermentation of 1-C^{14}-L-arabinose by *Lactobacillus pentoaceticus* and again ob-

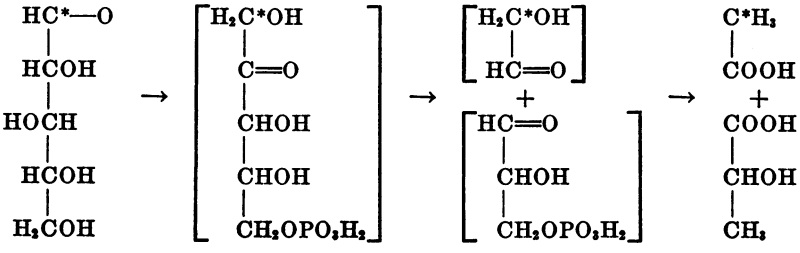

FIG. 5. Suggested mechanism of xylose fermentation by *Lactobacillus pentosus*

TABLE 1

Specificity of fermentation by Lactobacillus pentosus

CARBOHYDRATE IN MEDIUM	RATE OF SUBSEQUENT ACID PRODUCTION FROM			
	D-Glucose	D-Ribose	D-Xylose	L-Arabinose
D-Glucose	++++*	+	−	−
D-Ribose	++++	++++	−	+
D-Xylose	++++	++++	++++	+
L-Arabinose	++++	++++	−	++++

* Rates are graded from −, no fermentation, to ++++, maximum rate obtained with given cell type.

served that the aldehyde carbon of the sugar was the precursor of the methyl group of the acetic acid.

Since *L. pentosus* will grow on several of the C_5 sugars, it was of interest to determine whether the individual sugars were degraded by separate, though similar routes, or whether the pathways fused, as with many of the hexoses. We, therefore, determined the specificity of pentose fermentation by this organism with the results summarized in table 1. Each cell type ferments rapidly both glucose and the sugar present in the growth medium, that is, the cells grown on ribose ferment ribose and glucose, those grown on xylose ferment xylose and glucose, etc. The interesting feature, however, is the activation of the ribose-fermenting system by growth in the presence of the other pentoses. Thus growth on xylose or L-arabinose resulted in cells which fermented ribose rapidly, whereas

growth on ribose did not yield cells with significant activity on the other pentoses. It was also noted that extracts of cells grown on any of the three pentoses had an activity in degrading ribose-5-phosphate that was greatly increased over that of comparable extracts from cells grown on glucose. Xylose-5-phosphate was not metabolized by any of the extracts, nor was D-arabinose-5-phosphate. The obvious suggestion from these data is that D-xylose and L-arabinose may be converted to compounds with the ribose configuration before cleavage of the chain occurs. This would explain the increased activity of the ribose-metabolizing systems.

Guided by this hypothesis, we examined the initial reactions of pentoses in these organisms and were readily able to obtain extracts which catalyzed the phosphorylation of pentoses by ATP. The results are summarized in table 2. In analogy with the fermentation tests, each extract phosphorylated glucose and

TABLE 2

Specificity of phosphorylation by extracts of Lactobacillus pentosus

SUGAR IN GROWTH MEDIUM	PHOSPHORYLATION BY EXTRACT			
	Glucose	Ribose	Xylose	L-Arabinose
D-Glucose	++++*	+	±	−
D-Ribose	+++	++++	±	−
D-Xylose	++	++	++++	−
L-Arabinose	++++	++++	±	++

* Rates are graded from −, no phosphorylation, to ++++, maximum rate obtained with a given extract.

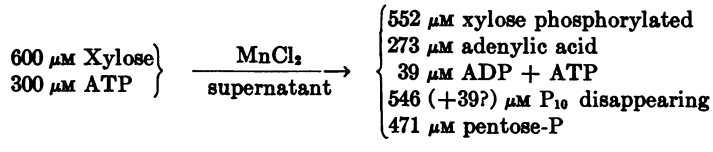

FIG. 6. Xylose phosphorylation in HCO_3—sonic extract

the sugar present during growth of the original cells. In addition, extracts of cells grown on xylose or arabinose catalyzed the phosphorylation of ribose, although with the extract of xylose cells the rate was slow. An extract of cells grown on ribose did not phosphorylate xylose or arabinose at detectable rates. This correlation between fermentation and phosphorylation indicates the phosphorylation of these sugars to be one of the first, though not necessarily *the* first step in their metabolism.

By the use of extracts prepared by sonic disintegration of the cells in bicarbonate buffer, rather than in the phosphate buffer used for the previous experiments, preparations were obtained which catalyzed the phosphorylation of the various sugars, but did not bring about any significant degradation of added ribose phosphates. Under these conditions direct testing of the proposed formation of ribose phosphate during xylose metabolism became feasible. Figure 6 summarizes

the data of a typical experiment. Xylose was incubated with ATP in the presence of an extract of *L. pentosus* which had been treated with $MnCl_2$ to remove most of the nucleic acid. The quantity of xylose phosphorylated was in good agreement with the values for acid-labile phosphate disappearing. The pentose phosphate was isolated as the barium salt and shown to contain pentose, reducing sugar, and organic phosphorus in approximately equimolar amounts. Chemical studies have shown that about 80 per cent of the product is ribose-5-phosphate. The rest of the material appears to be a ketopentose phosphate, although it has not been possible to identify the particular ketopentose present. Small amounts of ketoheptose phosphate, which Horecker and Smyrniotis (13) have shown to be formed during the metabolism of pentose phosphates in yeast extracts, appear to be present as well.

We can say then with assurance that we have demonstrated the main point at issue, *i.e.*, formation of phosphate esters with the ribose configuration from xylose and ATP. I should point out that ribose-5-phosphate, while it is the compound isolated in our system, may not be the intermediate involved in xylose degradation. Our isotopic experiments suggest a ketopentose intermediate, and it is to be expected that the formation of ribulose-5-phosphate in this system would result in the accumulation of ribose-5-phosphate since the pentose phosphate isomerase is present.

The mechanism of this transformation of xylose to a ribose ester is obscure at present. As mentioned previously, xylose-5-phosphate is inert in these systems, and we have been unable to obtain any evidence for the formation of acid-labile esters such as xylose-1-phosphate or ribose-1-phosphate. It is possible that xylose is converted to a compound with the ribose configuration, prior to phosphorylation of the sugar. Cohen[3] has demonstrated such a reaction during utilization of D-arabinose by *E. coli*, *i.e.*, the conversion of D-arabinose to D-ribulose. We have incubated xylose with our enzyme preparations in the absence of ATP and have observed the accumulation of a new sugar giving the orcinol test for pentose (24). Thus some interconversion of the free sugars may well be involved. Two observations suggest that ribose itself is not an intermediate in the xylose → ribose phosphate transformation: (a) cells grown on xylose and stored at 4 C for 4 days utilize xylose at an initial rate higher than that observed with ribose; (b) extracts of xylose-grown cells phosphorylate xylose in the presence of ATP at a rate several times that with ribose.

DECOMPOSITION OF NITROGENOUS BASES

I should like now to consider the fate of the nitrogenous bases formed by cleavage of the nucleosides. We have some insight into the complex transformations by which microorganisms degrade the purine ring structure. I will not discuss these except to note that Whiteley (30) has observed the formation of pyrimidines during the degradation of purines by *Micrococcus aerogenes*, demonstrating an interrelation in this organism, at least, between degradation of the two types of rings. To study the manner in which the pyrimidine derivatives

[3] Cohen, S. S. Personal communication.

accumulating in nature are decomposed by microorganisms, we obtained from soil by enrichment culture techniques a species of *Bacterium* which could utilize a pyrimidine as its sole source of carbon and nitrogen (28). No growth occurred with purines. Data on the oxidizability of various pyrimidine derivatives by this organism indicate that the initial oxidation by the intact cells occurs at position 6 of the pyrimidine ring, as is illustrated in figure 7. Compounds with a substituent on positions 3 or 5 can be oxidized, the latter, in fact, more rapidly than is uracil itself, but apparently one can make no alteration in the substituents at positions 1, 2 and 4 and still obtain an oxidizable compound. In these experiments with intact cells, the pyrimidine was oxidized to carbon dioxide, water and ammonia. No intermediate compounds could be identified in any significant quantity. With cell-free extracts, oxidation of uracil or thymine in the presence of methylene blue could be demonstrated (29). One atom of oxygen was consumed per mole of uracil, and the product of uracil oxidation was isolated and identified as barbituric acid.

Hayaishi (7) has observed the cleavage of barbituric acid in cell-free extracts of a *Mycobacterium* species. Urea and malonic acid were identified as the prod-

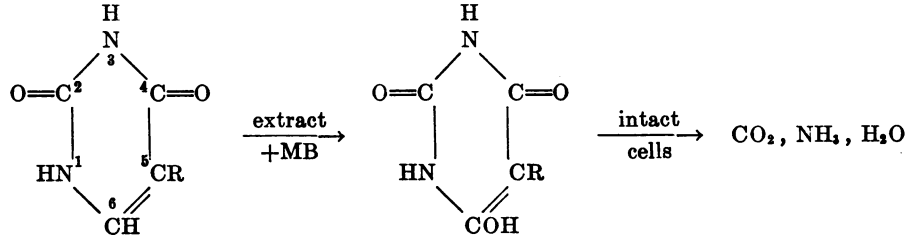

FIG. 7. Oxidation of pyrimidines by a *Bacterium* sp.

ucts. Our enzyme preparations have not catalyzed the further degradation of barbituric acid. Mr. M. Del Duca has recently obtained in our laboratory some data that may offer an explanation. He observed that the oxidation of barbituric acid by intact cells was prevented by the addition of 0.005 M methylene blue. Under these conditions oxidation of uracil to barbituric acid could be demonstrated. This inhibitory effect of methylene blue may be the basis for our inability to obtain extracts active on barbituric acid since methylene blue was the electron carrier in all of our experiments. This observation also strengthens the proof that the barbituric acid pathway is the chief mechanism of uracil oxidation in this organism.

FACTORS REQUIRED FOR GROWTH ON PENTOSES

The last subject I shall consider is that of the requirements of lactic acid bacteria for growth on pentoses. Several synthetic media have been described in which rapid and reproducible growth of the lactic acid bacteria is possible with glucose as the carbohydrate. During our investigations of pentose fermentation it appeared desirable to study the metabolism of organisms grown on synthetic

media containing pentose as the sole carbohydrate. In attempting such experiments it was observed that *L. pentosus* did not grow with xylose on a medium which was adequate for growth on glucose. As excessive charring occurs if pentoses are autoclaved with the constituents of the usual synthetic medium, it is necessary to autoclave these sugars separately and add them aseptically to the sterilized medium. Since many workers have reported stimulatory effects from autoclaved glucose, it appeared possible that a similar factor was involved here. However, by the addition of a reducing substance, such as cysteine, and further supplementation with B-vitamins, uracil, and Tween-80 a medium was obtained in which the rate of growth was essentially the same whether glucose was autoclaved with the medium or added aseptically. The organism was still unable to grow with xylose on this medium. The data presented in table 3 show that growth (as measured by turbidity) and acid production are excellent on glucose in the absence of a supplement, whereas neither growth nor acid production occurs with xylose unless a material such as liver extract or yeast extract is present.

TABLE 3

Growth requirements of Lactobacillus pentosus with glucose and xylose

SUGAR	LIVER EXTRACT	TURBIDITY IN 40 HR	ACID (ML, 0.1 N) IN 90 HR
None	−	0.0*	0.0
	+†	0.07	0.10
D-Glucose	−	0.72	3.20
	+	0.91	3.30
D-Xylose	−	0.01	0.10
	+	0.60	3.05

* Optical density.

† 0.7 mg per ml (total volume of medium 3.0 ml).

The liver extract supplement supports relatively little growth in the absence of the sugar.

This substance(s), which we have termed the *Pentose Factor*, is required for growth or xylose, ribose, and arabinose, and also with glycerol, but is not essential for growth with glucose, mannose, or fructose. It is synthesized in rather large amounts during growth of *L. pentosus* on glucose, but no synthesis was detected during growth of the organism on xylose with a suboptimal quantity of the factor. This indicates that the factor is not a substance uniquely required in the metabolism of pentoses, but rather that it is generally essential for growth and can be formed by the cell in adequate quantities only from the hexoses. It has not been possible to identify it with any of the known purified growth factors.

The requirement of this factor by lactic acid bacteria appears to be rather general, although specific conditions may be required for its demonstration. Thus *L. pentosus* when tested in mass liquid culture will often grow, after a lag, on ribose or arabinose in the absence of the factor. This difficulty can be circum-

vented by plating known numbers of viable cells with the various sugars in the presence or absence of the supplement (see table 4). Under these conditions the colonies develop rapidly with the supplement, whereas in the absence of liver extract only a few colonies develop after two or three days of incubation, and these colonies on subculture grow readily in the absence of the factor. The requirement of this supplement for growth by the bulk of the population therefore is definite. Similar results were obtained with the other homofermentative organisms listed, *i.e.*, *Lactobacillus arabinosus* and *Streptococcus faecalis* R. An interesting observation was that the heterofermentative organism, *Lactobacillus pentoaceticus*, requires the addition of liver extract even in the presence of glucose. Since this organism has been shown (2) to metabolize glucose by a path other than the Meyerhof system of the *homofermentative* organisms, this requirement is

TABLE 4

Growth requirements in plate tests

ORGANISM	LIVER EXTRACT	SUGAR			
		Glucose	Ribose	Xylose	L-Arabinose
Lactobacillus pentosus	−	4+	−	−	−
	+	4+	4+	4+	4+
Lactobacillus arabinosus	−	4+	−		−
	+	4+	4+	−	4+
Streptococcus faecalis R	−	4+	±*		
	+	4+	4+	−	−
Lactobacillus pentoaceticus	−	−	−	−	−
	+	4+	4+	3+	4+

* Requirement for "Pentose Factor" lost rapidly.

additional evidence that the factor in question is synthesized during the early stages of glucose metabolism.

Two reports in the literature appear rather significant in relation to this factor. Heald (8) stated recently that several strains of *Escherichia coli* 'intermediates' formed C_4 compounds such as acetylmethylcarbinol and 2,3-butylene glycol in good quantities from glucose but did not make these in detectable amounts from pentoses. He suggested that a cofactor required for the formation of these C_4 compounds was produced during the metabolism of glucose, but not of xylose. Some years ago Reynolds and Werkman (27) observed that a strain of *E. coli* produced CO_2 and H_2 in large quantities during the anaerobic decomposition of glucose, but produced essentially none of these gases from xylose. Since Lascelles (19) has reported a stimulatory effect of glucose on hydrogenlyase production, it is possible that the cells grown with xylose are deficient in this enzyme. The recent demonstration by Lichstein (20) of a cofactor requirement for formic hydrogenlyase is consistent with this concept and suggests a relation to the factor

we have been studying. It is obvious that further experimentation is necessary before we can go beyond this comparison.

Before closing I should like to reemphasize my debt to those who have aided in these investigations. Doctors Gest, Hoffmann, Manson, Roepke, and Wang have been particularly concerned. With the complex nature of modern research, one can only rarely work completely alone, and I am convinced that the work discussed in this review would have faltered many times without their sincere cooperation.

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